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ORIGINAL RESEARCH

Zingerone Attenuates Cadmium-Induced Neuroinflammation, Oxidative Stress and Cognitive Deficit on the Prefrontal Cortex of Adult Wistar Rats

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Introduction/Background: Exposure to heavy metals like cadmium can adversely affect our brain function and cognitive abilities. Scientific evidence has strongly linked cadmium exposure to neurotoxicity, including oxidative damage and neuroinflammation. This study investigated the therapeutic role of *Zingerone* on cadmium-induced inflammation, oxidative stress and cognitive decline in the prefrontal cortex.

Methodology: Thirty adult male Wistar rats were randomly assigned to six groups of five rats each. Group A (Control group), Group B (5mg/kg of cadmium induction), Group C (100mg/kg of *Zingerone* only), Group D-F (Treatment groups: 5mg/kg of cadmium +50mg/kg,100mg/kg, 200mg/kg of Zingerone respectively). To determine the cognitive abilities, we used a novel object recognition test (NORT), the whole treatment lasted for 21 days. We used blood samples and brain tissue for histological, biochemical and immunohistochemical evaluations. Data from this study was analysed using GraphPad Prism 10, and statistical difference between groups was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$).

Result and discussion: There was significant reduction in superoxide dismutase (SOD), catalase (CAT) and significant increase malondialdehyde (MDA) in the cadmium-only group; however, this effect was mitigated in Groups C–F that received zingerone treatment. Cadmium exposure resulted in elevated levels of inflammatory cytokine Interleukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-α), these increases were significantly reduced in all *Zingerone-treated* groups. *Zingerone* considerably improved cognitive function, as seen by significant improvements in the discrimination index and novel object recognition time. Also, *Zingerone* significantly mitigated the over-expressivity of astrocytes in the prefrontal cortex and improved the histoarchitecture of the prefrontal cortex.

Conclusion: This study shows that *zingerone* demonstrated anti-oxidative and anti-inflammatory abilities and enhanced cognitive performance. These results indicate that zingerone might be a useful therapeutic agent that can mitigate neuroinflammation, oxidative damage, and cognitive decline.

Keywords: astrocytosis, neuroinflammation, oxidative stress, cognitive impairment, zingerone, cadmium

Introduction

Scientific data has established a connection between cognitive deficits and prolong exposure to environmental toxins like heavy metals, pesticides, plastics and mycotoxins.[1] Environmental pollution has been linked to neurodegenerative disorders and cognitive impairments. Various studies have investigated the mechanism of environmental toxins-induced

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cognitive function, with some research indicating that elevated levels of cadmium, particularly in the elderly, may be detrimental to cognitive health.[2–4] Cadmium, an heavy metal released during the smelting of copper, zinc, lead, and other metals.[3–5] Humans may get exposed to cadmium through contaminated food or drink, skin absorption, occupational contact, and tobacco smoke inhalation.[6,7] The International Agency for Research on Cancer (IARC) has classified cadmium as a Group 1 carcinogen, indicating that it is an airborne pollutant that constitutes a significant danger to human health.[8–13]

Cadmium (Cd) is a common environmental contaminant that has been connected to the pathophysiology of several neurodegenerative diseases because of its strong pro-inflammatory and neurotoxic effects.[6,13] Cadmium mainly enters the central nervous system via oral cavity, where it enters the bloodstream, interferes with the blood-brain barrier (BBB) and builds up in the tissue of the nervous system. Cadmium can enter brain tissue directly through inhalation from the olfactory pathway or nasal mucosa, since the Blood Brain Barrier (BBB) does not protect the olfactory epithelium.[14,15] Studies have shown that exposure to Cd at doses ranging from 0.44 mg/kg b.w. to 200 mg/kg b.w. Causes systemic oxidative stress, tissue inflammation, and ultimately tissue damage, which impacts several organ systems.[6,14]

Prolonged exposure to cadmium has been specifically associated with progressive loss in memory, cognitive decline and an increase in the onset of neurodegenerative diseases.[11] The prefrontal cortex and hippocampus are responsible for complex cognitive behaviour and decision-making, and both the hippocampus and prefrontal cortex are adversely affected by cadmium exposure.[6,11,13] Cadmium exposure triggers oxidative stress, mitochondrial dysfunction, inflammation, death of neurons and glial cell activation in the prefrontal cortex and the hippocampus.[6,7] One mechanism by which cadmium mainly induces toxicity and oxidative stress in brain cells is by generating reactive oxygen species (ROS) and consequently decreasing intracellular glutathione (GSH), catalase, and SOD activity.[14–23]Exposure to cadmium induces inflammation, and numerous studies on the pro-inflammatory effects of cadmium have shown that it increases the production of IL-6, TNF-α, and IL-1 both in vitro and in vivo. Cadmium exposure triggers oxidative stress and systemic inflammation, which damage the blood-brain barrier, encourage neuroinflammation, and increase the risk of brain toxicity and cognitive decline.[6,17–21]

Medicinal plants and bioactive components are being utilised increasingly for the management of neurodegenerative diseases like Parkinson's, Alzheimer's, and dementia, and to mitigate neurotoxicity.[24–28] (Zingerone (4-(4-hydroxy-3-methoxyphenyl)-2-butanone), a substance found in ginger, has several pharmacological actions, which include anti-oxidative, anti-inflammatory, hepato-protective, anti-cancer, anti-microbial, anti-diabetic, nephroprotective, and neuro-protective properties. [29–32] Zingerone is a crystalline material with a molecular mass of 194.22 g/mole, a melting and boiling point of 40–41 and 187–188 degrees Celsius, respectively. Its structural and chemical formula is $C_{11}H_{14}O_3$.[29,33] Due to its strong anti-inflammatory and antioxidant properties, several medical conditions, including diabetes, cancer, renal damage, cardiovascular disease, arthritis, Alzheimer's disease, Parkinson's disease, and cognitive impairment, may benefit from zingerone.[29,31,34–39]

Despite growing knowledge of the harmful effects of cadmium exposure on the central nervous system, particularly the prefrontal cortex, which regulates cognition and decision-making, there are still few effective alternatives to treatment currently. Zingerone, a bioactive compound derived from ginger (Zingiber officinale), is known for its anti-inflammatory, antioxidative and neuroprotective properties.[31,32,36–38] However, its specific role in mitigating cadmium-induced neuroinflammation and cognitive dysfunction in the prefrontal cortex has not been thoroughly investigated. This study is therefore novel in its focus on evaluating the therapeutic potential of zingerone against cadmium toxicity in a targeted brain region critical for higher-order functions. Zingerone's neuroprotective efficacy is, to an extent, dependent on its ability to cross the compromised BBB in cadmium-exposed rats. This study is additionally vital because it might be used as a basis for developing affordable, plant-based therapy for the management of environmental neurotoxic exposure. Thus, this work aims to investigate the anti-inflammatory and anti-oxidative effects of zingerone on cadmium-induced neurodegeneration and cognitive impairment in adult male Wistar rats.

Materials and Methods

Chemical and Reagent

All of the reagents utilised in this investigation, including zingerone, commonly referred to as vanillyl acetone (CAS NO: 122–48-5, with purity ≥98%) and cadmium (CAS NO: 7440 439), were obtained from the USA, Sigma Aldrich. These products were confirmed to be of analytical standard with a valid shelf life.

Experimental Design/ Grouping

In this study, we obtained 30 adult male Wistar rats (10–12 weeks old, weighing 140–200g) from the animal farm of the University of Nigeria, Nsukka. The rats were kept in clean, well-ventilated metal cages in the Anatomy Department animal house facility. The laboratory animals were kept at a regulated temperature and subjected to 12 hours of light/dark cycles and allowed free access to water and standard food. The rats were assigned randomly into six groups (n=5) as shown in Table 1. Group A; (Normal Control) received normal saline, Group B, (Cadmium Only) was administered cadmium (5mg/kg) orally for 7 days, Group C; (Zingerone Only) received 100 mg/kg zingerone orally for 14 days, Group D; Cadmium(5 mg/kg) for 7 days and 50 mg/kg zingerone for 14 days, Group E Cadmium(5 mg/kg) for 7 days and 100 mg/kg zingerone for 14 days, Group F; Cadmium(5 mg/kg) for 7 days and 200 mg/kg zingerone for 14 days. We dissolved zingerone and cadmium in normal saline and administered them orally using an oral cannula. Daily assessments of the rats' weight, cage activities, and physical appearance were recorded. The Novel Object Recognition Test (NORT), as described by,[40] was used to assess cognitive ability. Cadmium chloride was administered, and zingerone was administered orally, with dosage determined based on previous studies.[39,41,42] Cadmium administration for all groups was for 7 days, and zingerone was administered for 14 days, as shown in Table 1.

Ethical Approval

The research proposal was presented and approved by the Faculty of Basic Medical Science, College of Medicine Research Ethics Committee, University of Nigeria, Enugu Campus (UNN/FBS/REC). The current study adhered to the ARRIVE guideline and the Institutional Animal Ethics Committee, USA (IAEC) of the National Institutes of Health's regulations and standards regarding the care of animals.

Novel Object Recognition Test (NORT)

The NORT is a widely recognised neurobehavioral model used to evaluate learning, cognitive ability and retention in experimental animals.[40,43] It assesses working memory, attention, alternations, and novelty preference by exposing animals to familiar and novel objects.[44] In this study, the NOR test apparatus used consisted of a square wooden box (100x100x100 cm) with an open field for object placement. We carried out a baseline test immediately after acclimatising the animals before the commencement of treatment. Thereafter, after administration on the last day of the experiment period, we conducted a novel object test. We followed the standard NOR test procedure according to.[45] The test was divided into three stages: familiarisation, training, and the test phase, and the duration of the test was five minutes. In the training phase, the rats freely explored two similar objects and familiarised themselves with them for 5 minutes. During the test phase, we replaced one of the previously explored objects with a new one of similar size and shape but a different

Table 1 Table Showing Experimental Design/ Grouping

Group	Substance Administered /Dose	Duration
A	Normal saline	21 days
B	5mg/kg Cadmium chloride only	5mg/kg $Cdcl_2$ for 7 days
C	100mg/kg Zingerone only	100mg/kg Zingerone 14 days
D	*5mg/kg Cadmium chloride + 50mg/kg zingerone	5mg/kg $Cdcl_2$ for 7 days + 50 mg/kg zingerone for 14 days
E	*5mg/kg Cadmium chloride + 100mg/kg zingerone	5mg/kg $CdCl_2$ for 7 days +100mg/kg zingerone for 14 days
F	*5mg/kg Cadmium chloride + 200mg/kg zingerone	5mg/kg $Cdcl_2$ for 7 days + 200mg/kg zingerone for 14 days

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colour. The animals explored both objects for 5 minutes, and the time use in exploring the familiar and novel objects was properly recorded. The behavioural procedure was recorded with a digital camera, and data were recorded for analysis.

The time spent (seconds) examining the new object in comparison to the familiar object and the discriminating index was used to evaluate novel object memory. An animal's preference for a novel object over a familiar one is determined by the discrimination index (DI) in a novel object test.

The discrimination index helps to evaluate exploratory behaviours and recognition memory.The Discrimination Index formula is: $DI = Tn + Tf/Tn + Tf$

In Time, exploring novel objects and Tf time exploring familiar objects.

Animal Sacrifice and Collection of Samples

The experimental rats were weighed using a digital weighing scale and sacrificed via cervical dislocation under light chloroform anaesthesia on day 22, twenty-four hours after the treatment and behavioural test period. Samples of the brain were carefully removed, weighed, and preserved in 10% neutral buffered formalin. We obtained blood samples carefully via retro-orbital puncture on the superior orbits of the rats. Blood and brain tissue samples were sent to the laboratory for biochemical, immunohistochemical, and histological analysis.

Biochemical Assay: Measurement of Inflammatory Cytokines (IL-6 and TNF-α)

(TNF-α) and (IL-6) are significant cytokines associated with inflammatory response. We evaluated the levels of TNF-α and IL-6 in serum samples using commercially available BioLegend ELISA kits (Catalogue Numbers: 430504 for IL-6 and 430204 for TNF-α) following the manufacturer's instructions and the procedure outlined by[46,47] Results were expressed in ng/mL.

Measurement of Antioxidant Enzyme Activity and Oxidative Stress Markers

Estimation of Superoxide Dismutase (SOD) Activity

The Marklund & Marklund's approach was utilized to measure the level of superoxide dismutase (SOD). The prefrontal cortex of the brain was immediately frozen and homogenised using routine procedures.[30] 0.1 mL of 30 µM EDTA, 0.1 mL of the enzyme extract, and 2.5 mL of 0.05 M sodium carbonate buffer (ph 10.2) made up the reaction mixture. After adding 0.3 mL of freshly made 0.3 mm epinephrine to start the process and the increase in absorbance was observed at 480 nm for 5 minutes. The quantity of enzyme needed to produce a 50% inhibition of epinephrine auto-oxidation was determined to be one unit of SOD activity. SOD levels were expressed as u/mg protein.[48]

Estimation of Malondialdehyde (MDA) Activity

Using the thiobarbituric acid reactive substances (TBARS) approach, as outlined by Ohkawa et al, the MDA level was calculated to detect lipid peroxidation.[49] We homogenised the prefrontal brain tissues using a homogeniser following the standard procedure. To estimate MDA activity, 0.5 mL of tissue homogenate, 1 mL of 0.67% thiobarbituric acid (TBA), and 1 mL of 20% trichloroacetic acid (TCA) were mixed. The mixture was centrifuged for 10 minutes at 3000 rpm after being heated for 15 minutes at 95°C in a boiling water bath. At 534 nm, the absorbance of the pink MDA–TBA combination in the supernatant was determined. MDA concentration of 1.56×10^5 M^1 cm^1 was determined using a molar extinction coefficient. MDA activity was expressed as µmol/µg protein.

Estimation of Catalase (CAT) Activity

In compliance with Claiborne method, catalase (CAT) activity was investigated using the homogenised brain tissue. For a total volume of 3.0 mL, the assay mixture included 0.1 mL of sample, 10 mm H_2O_2, and 50 mm phosphate buffer (ph 7.0). The reduction in absorbance at 240 nm over 60 seconds was used for monitoring the breakdown of H_2O_2. Using a molar extinction coefficient of 43.6 M^{-1} cm^{-1}, catalase activity was calculated and represented in u/mg protein.[50]

Histopathological Examination

After sacrificing the animals, we used transcardiac perfusion to infuse sterile Phosphate Buffered Saline (PBS) into the animals. The brain was carefully removed, rinsed with normal saline solution, and fixation was done using 10% neutral buffered formalin. After that, the brains were processed histologically following the standard documented histological methods,[30] which include: Clearing, Infiltration, Dehydration, Embedding, Sectioning and Staining with Cresyl Fast Violet (CFV) and Hematoxylin and Eosin (H&E). An Olympus CX31 binocular light microscope (Olympus USA) connected to a digital camera was used for examining the stained sections, and photomicrographs were captured at magnifications of X40, X400.

Immunohistochemistry (GFAP)

Thin sections of the medial prefrontal cortex (mpfc) area were subjected to GFAP immunostaining to visualise specific protein expressions through antigen-antibody reactions. Astrocyte expression in the prefrontal cortex was quantified using Image J Software according to methods described by.[51]

Light Microscopy and Cell Count

Using an Olympus CX31 binocular light microscope (Olympus, New Jersey, USA) coupled to a 5.0 MP AmScope digital camera (AmScope Inc., USA), photomicrographs of the prefrontal cortex's histology and immunohistochemistry were captured. Image-J software (NIH, USA) was used to determine the cellular immunopositivity, cell count and histochemical labelling. Using the built-in "multi-point" feature, the number of GFAP-positive astrocytes (those whose nucleus was visible) in each image was manually counted. The result was then translated to counts per mm^2. A X40 and 400x magnification was used to take each photograph. To get the average count per mm^2, we divided the total counts for a region by the total area. To keep all samples consistent, ROIS were standardised to be 200×200 μm (40,000 μm^2) in size. Blinded counting was done to prevent bias and ensure objectivity. This method made it feasible to quantify the astrocyte density in the PFC with accuracy and consistency.

Data Analysis

Quantitative data from NORT behavioural tests and pro-inflammatory markers were analysed using GraphPad Prism version 10. Statistical significance was determined using one-way ANOVA and Tukey's Post Hoc test, with $p < 0.05$ considered statistically significant. Bar charts were used to present the results, illustrating the mean and standard error of the mean.

Result

Result of Body Weight of Animals

The study examines their final body weight, initial body weight, and corresponding weight change of the animals in Figure 1. To determine the impact of the experimental conditions on the growth and development of the animals, these factors were systematically assessed.

Our results indicates that there was an increase in the weight of the rats across the groups when the initial body weight was compared to the final body weight. However, the weight gain showed that the normal control group,and zingerone-only treated group had significantly higher weight gain compared to the 5mg/kg cadmium alone group. The cadmium-only group showed a minimal weight gain, while zingerone treated groups (D-F) showed normalcy in weight gain as shown in Figure 1.

Effect of *Zingerone* on Cognitive Ability and Memory Using Novel Object Test (NORT)

Figures 2 and 3a–c illustrates the result of the neurobehavioral test. Figure 2 shows the data from the baseline test before commencement of treatment, while Figure 3 shows the NORT data at the end of the last treatment week. Using the Novel Object Recognition Test, the animals' preference for new objects over familiar objects was assessed, as well as the discrimination index. Time spent exploring novel objects was compared with time spent exploring previously familiar

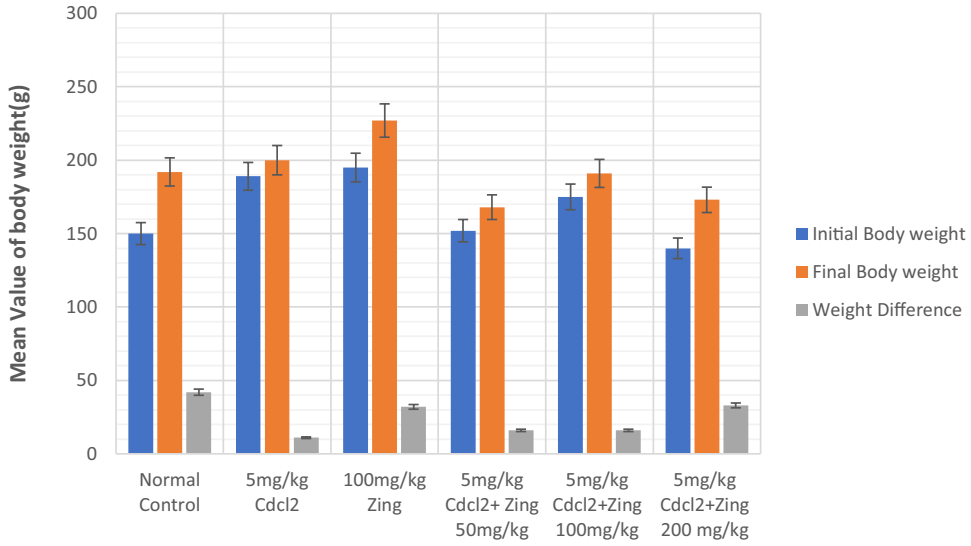

Figure 1 Comparison of initial, final body weights and weight difference across the experimental groups. Bar chart showing mean ± SEM of initial and final body weights, along with calculated weight differences, in six experimental groups.
Abbreviations: Zing, *zingerone*; CdCl2, cadmium chloride.

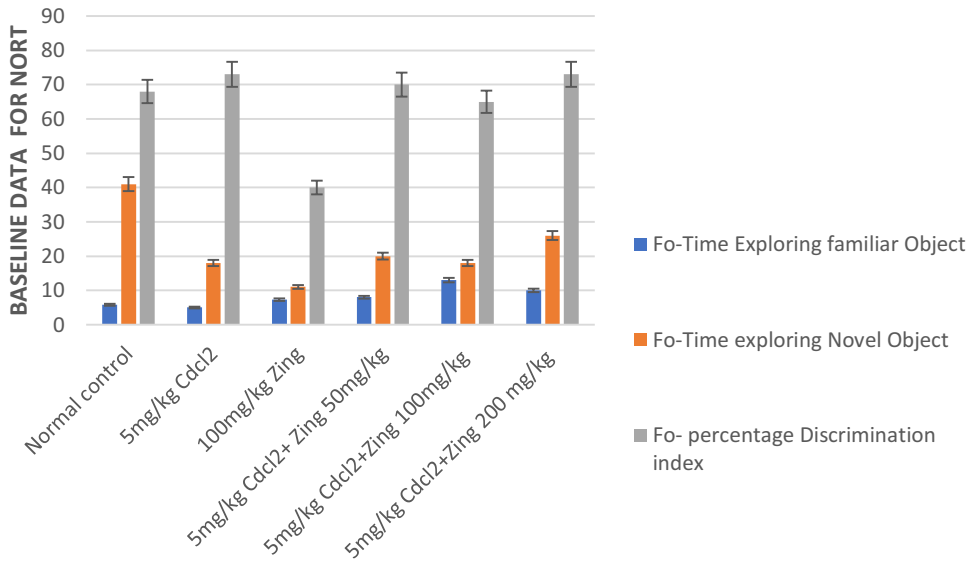

Figure 2 Bar chart illustrating the baseline data obtained from the Novel Object Recognition Test (NORT) across different treatment groups. Fo-Time Exploring Familiar Object (blue): The duration animals spent exploring a familiar object during the test. Fo-Time Exploring Novel Object (Orange): The time spent exploring a novel object (in seconds). Fo-Percentage Discrimination Index (grey). Value expressed as Mean±SEM.

objects. The results showed that Group B's cadmium administration significantly ($p < 0.05$) decreased the amount of time spent investigating novel objects when compared to the other zingerone treatment groups and the normal control group. In contrast, the administration of zingerone significantly increased the duration spent exploring the novel object and Discrimination Index as seen in Groups E and F, and also significantly improved the discrimination index in all zingerone-treated groups..

Neuroinflammatory Role of Zingerone (IL-6 and TNF-α)

In Figure 4a and b, the result of biochemical analysis of pro-inflammatory markers showed a significant elevation in IL-6 and TNF-α ($p < 0.05$) in the group administered with 5mg/kg of cadmium in comparison to both the normal control and the treatment groups. This confirms that exposure to cadmium significantly increases pro-inflammatory markers.

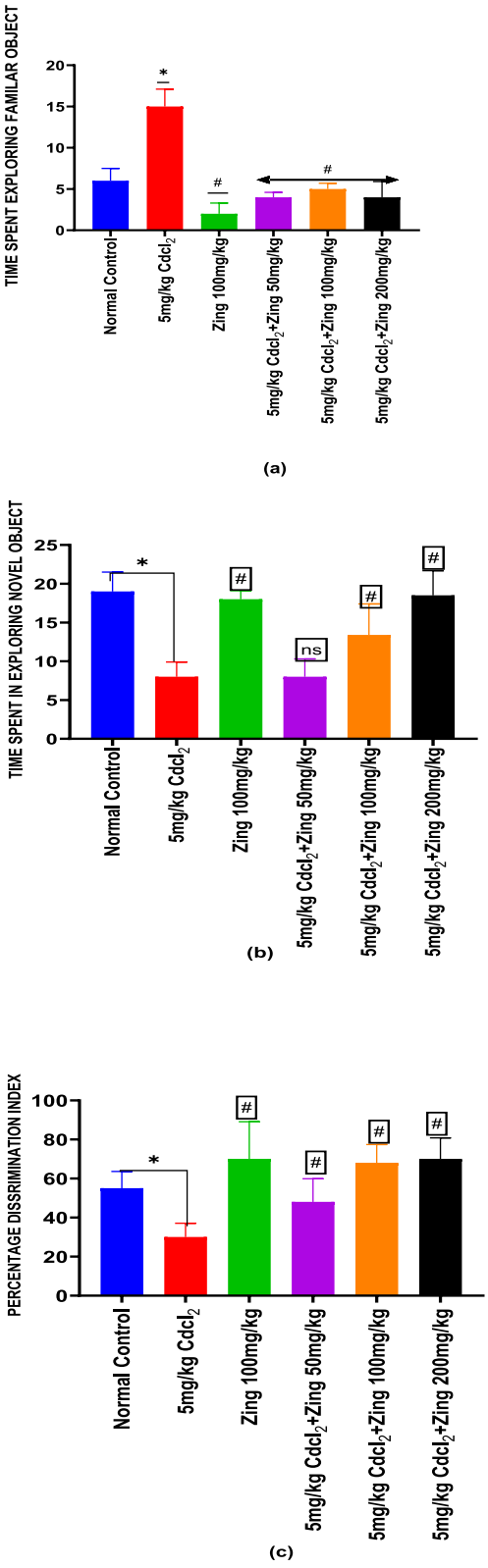

Figure 3 (**a**–**c**) Bar charts showing Novel object recognition test (NORT) on the Final week. (**a**) Time exploring familiar object(seconds), (**b**) Time exploring novel object (seconds), (**c**) Discrimination index percentage. *Represents statistically significant difference from the normal control group ($P < 0.05$), #Represents statistically significant difference in comparison to the cadmium only group ($P < 0.05$). Data expressed as Mean ± SEM. (n=5 rats).
Abbreviations: Zing, Zingerone; $CdCl_2$, Cadmium chloride.

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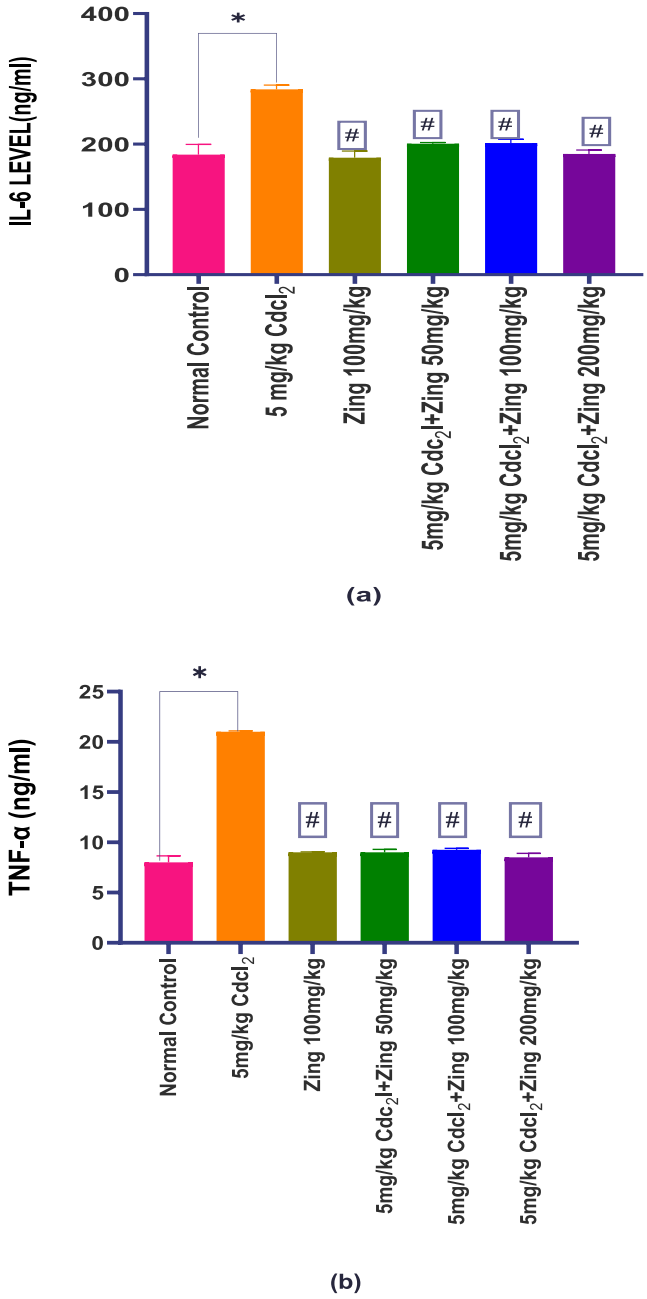

Figure 4 (**a** and **b**) Bar charts showing mean value for inflammatory cytokines. (**a**) IL-6 (**b**) TNF-α. *Represents statistically significant difference from the normal control group ($P < 0.05$), #Represents statistically significant difference in comparison to the cadmium only group ($P < 0.05$). Data expressed as Mean ± SEM. (n=5).

However, in the treatment group, our findings showed that zingerone treatment significantly attenuated IL-6 and TNF-α compared to the cadmium-only group.

Anti-Oxidative Role of Zingerone (SOD, MDA, CAT)

Figure 5a–c shows the result of oxidative stress markers (SOD, CAT, MDA) levels. The result of biochemical analysis of oxidative stress markers showed that there was a significant reduction in CAT and SOD activity ($p < 0.05$) in the group exposed to 5mg/kg of cadmium alone compared to the normal control group and the zingerone treatment groups. In addition, we observed that group B, which received 5 mg/kg of cadmium alone, had a noticeably higher MDA level than

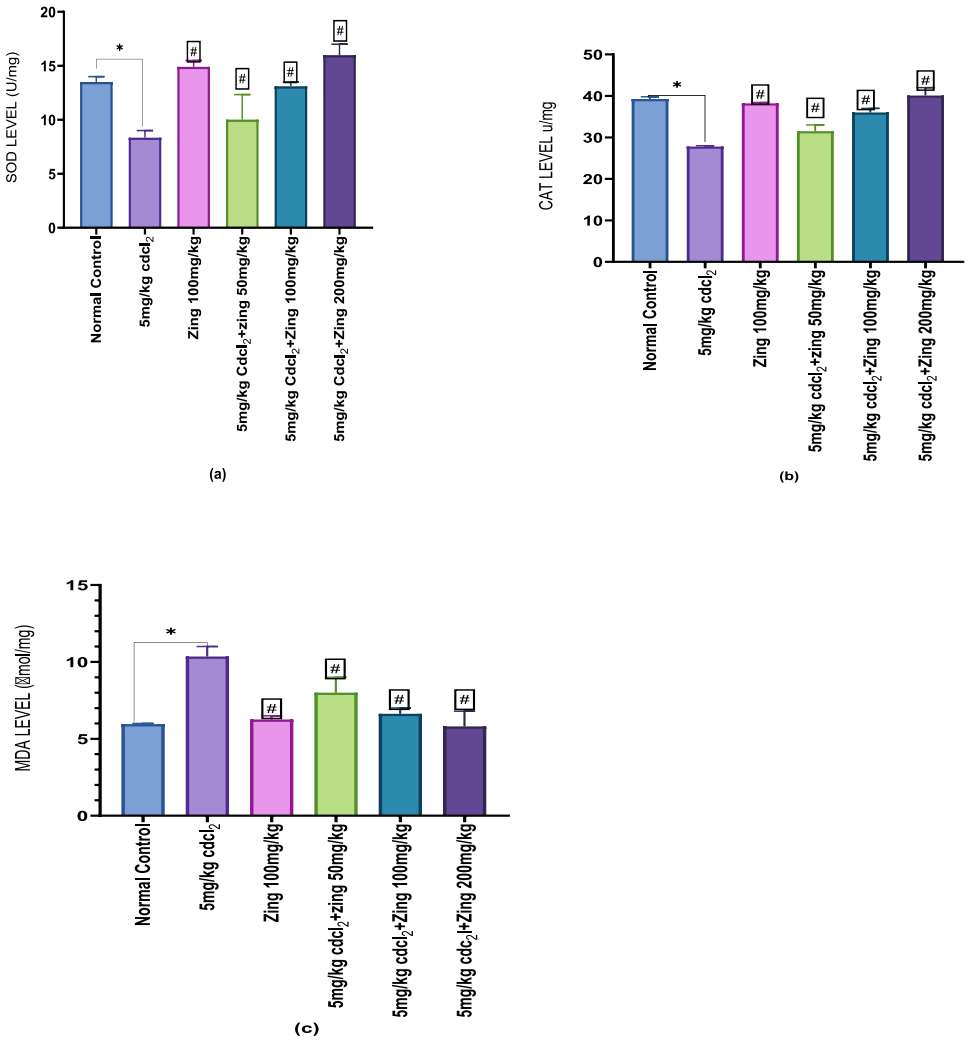

Figure 5 (**a**–**c**) Bar charts showing mean values for anti-oxidant enzymes and oxidative stress markers. (**a**) SOD level (**b**) CAT level (**c**) MDA level. *Represents statistically significant difference from the normal control group ($P < 0.05$), #Represents statistically significant difference in comparison to the cadmium only group ($P < 0.05$). Data expressed as Mean ± SEM. (n=5 rats).

the groups that received zingerone and the normal control. This confirms that cadmium exposure triggers oxidative stress, which ass significantly attenuated by zingerone.

Effect of Zingerone on Histology and Immunohistochemistry (GFAP) of the Prefrontal Cortex

Figures 6 and 7 show histology of the prefrontal cortex using H &E (magnification 40 and 400).

The normal control group (A) showed a normal prefrontal cortex morphology. Major histological changes, including pyknotic neurons, fragmented cytoplasm, and chromatolysis, were observed in Group B, which received only 5mg/kg of cadmium. In comparison to group C (100mg/kg of Zingerone only), the prefrontal cortex showed a normal histology. Groups D and E, which were administered cadmium and administration with zingerone (50 mg/kg, 100 mg/kg), respectively, showed mild histological alterations. Group F, which received 5mg/kg of cadmium and was administered with zingerone (200 mg/kg), exhibited normal prefrontal cortex histology with no signs of cytoplasmic fragmentation, pyknotic nuclei, or chromatolysis.

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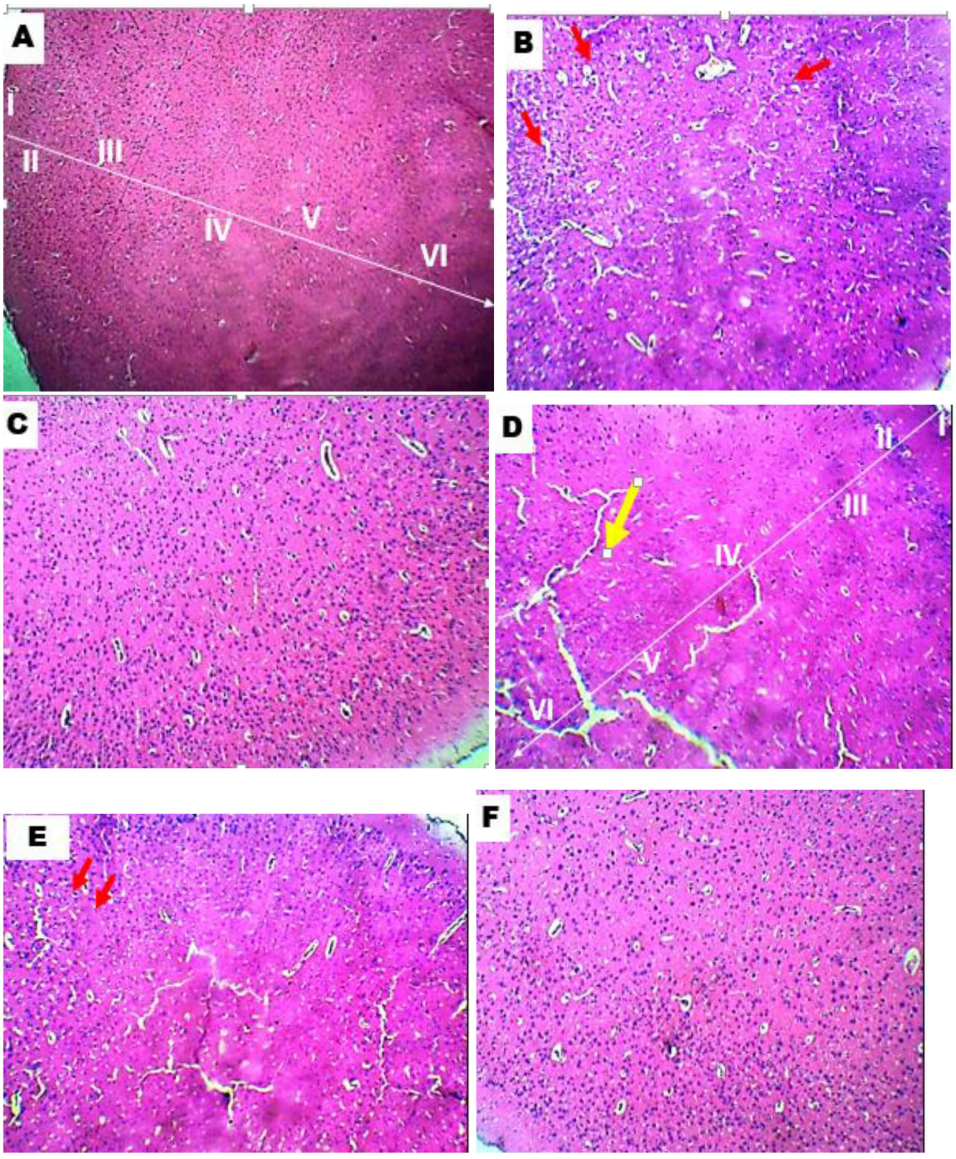

Figure 6 (**A**–**F**): Photomicrographs showing general histology of the prefrontal cortex of rats in group A-F.(H&EX40). The molecular layer (I), External granular layer (II), External pyramidal layer (III), Internal granular layer (IV), Internal pyramidal layer (V) and the multiform layer (VI) are demonstrated across study groups (white arrow direction i–vi). (**A**) normal histology of the Prefrontal cortex. (**B**) histology of the prefrontal cortex characterised by clusters of pyknotic pyramidal and granule neurons with appearance of fragmented cytoplasm as indicated by the red arrow. (**C**) All layers of the prefrontal cortex are seen with a normal histology. (**D** and **E**) appear mildly normal with arrows indicating slight alteration in the pyramidal cells (yellow and red arrow). (**F**) showed a normal histology of the prefrontal cortex, with no visible sign of pyknotic neurons and normal cytoplasm. (scale bar: 50 μm).

Figures 8 A-F and 9A–F: Showing Histology of the Prefrontal Cortex Using Nissl Staining (CFV)

In Figures 8 and 9, using CFV stain to visualise the histology of the prefrontal cortex, shows a normal histology of the PFC in the normal control group, with normal appearance of the neurons and Nissl substance. However, cadmium administration in group B showed diffused neurons, chromatolysis with reduced Nissl substance and loss of cellular processes. In the treatment groups C-F, results showed that rats administered with zingerone only and post-treated with zingerone showed no sign of pyknosis or chromatolysis, as the layers of the prefrontal cortex were seen with no sign of diffused neurons, especially observed in the group administered with a higher dose (200mg/kg) of zingerone.

Figure 10 shows the photomicrographs of the immunohistochemical examination of the prefrontal cortex across Groups A-F, also Figure 11 highlighted quantitative analysis of GFAP expression. The results indicated decreased

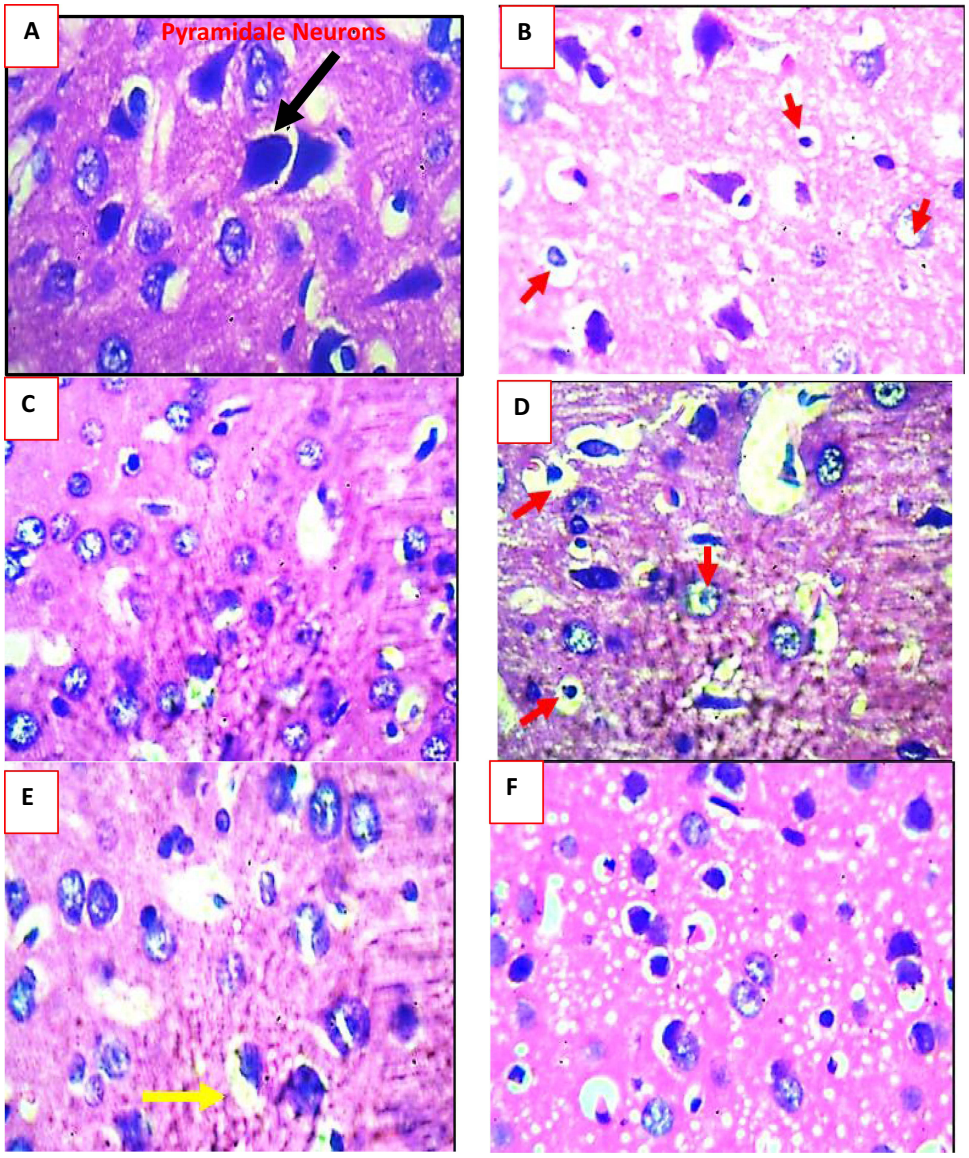

Figure 7 (**A**–**F**) Presents photomicrographs depicting the general histological architecture of the prefrontal cortex (PFC). Hematoxylin and Eosin (H&E) at ×400 magnification. In Group (**A**) PFC exhibits normal histology, with well-preserved cortical layers and intact pyramidal and granule neurons as indicate by arrow. (Group **B**) shows significant histopathological alterations, including clusters of pyknotic pyramidal and granule neurons, characterised by fragmented cytoplasm and condensed nuclei, indicative of neurodegenerative changes, red arrow showing pyknotic pyramidal neurons.(Group **C**) Displays normal neuronal morphology, with no observable signs of degeneration, (Groups **D** and **E**) Reveal mildly altered histoarchitecture; although the general organisation is preserved, there are subtle morphological changes in pyramidal neurons, as indicated by arrows red and yellow respectively, suggesting early or mild neurotoxic effects. (**F**) (Group **F**) demonstrates normal histological features, with no visible evidence of pyknosis or cytoplasmic damage, and neurons are well-organised.

astrocyte activity in the normal control group compared to the high GFAP expressivity in the group administered with 5mg/kg of cadmium only. Groups C-F showed significantly reduced astrocyte expressivity compared to Group B, suggesting that zingerone treatment mitigated astrocyte activation in response to cadmium-induced toxicity.

Discussion

Environmentally induced neurodegeneration and neurotoxicity are a major health care challenge. Exposure to environmental toxins like cadmium has been reported to cause neurological disorders and cognitive decline.[21,52,53] Severe exposure to Cadmium (Cd) has been linked to neurotoxicity as a result of its capability to cross the blood-brain barrier (BBB). According to studies, Cd can impair BBB integrity by causing oxidative stress, which disrupts tight junction proteins like zonula occludens-1 (ZO-1) and vascular endothelial cadherin (VE-cadherin).[16,54–56] This disruption

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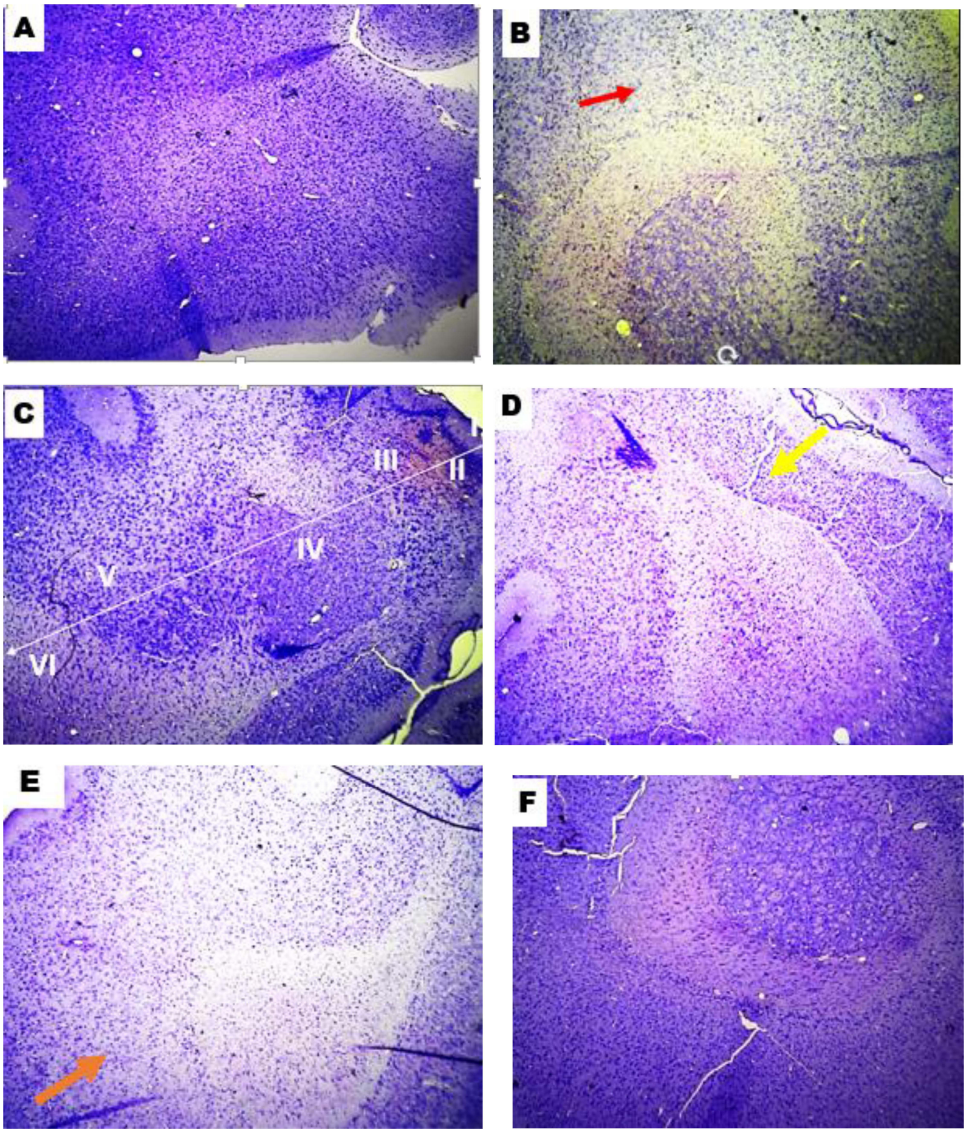

Figure 8 (**A**–**F**) Photomicrographs of the prefrontal cortex of rats (**A**–**F**). The layers of the prefrontal cortex are demonstrated by (CFV) Nissl staining (X40). The molecular layer (I), External granular layer (II), External pyramidal layer (III), Internal granular layer (IV), Internal pyramidal layer (V) and the multiform layer (VI) are demonstrated across study groups (white arrow). (**A**)Normal histoarchitecture of prefrontal cortex, (**B**) there were observable diffused neurons, chromatolysis with reduced Nissl substance and loss of cellular process indicated by red arrow. C-showed a normal histoarchitecture of the prefrontal cortex, Nissl profile appeared intact with no sign of chromatolysis, no sign of pyknosis. (**D** and **E**) showed a slightly normal histoarchitecture of the Prefrontal cortex, yellow and red arrows respectively show signs of diffuse neuron and chromatolysis. (**F**) Normal histoarchitecture of prefrontal cortex with no sign of chromatolysis, no sign of pyknosis. (Scale bar: 50 μm).

promotes BBB permeability, which allows Cd to penetrate the brain and contributes to neurodegenerative diseases. Furthermore, Cd has been demonstrated to activate caspase-3 and pannexin-1 channels, aggravating BBB failure and causing neuroinflammation.[57]

There is an increasing need for more research toward finding the safest, effective medication for the management of dementia and neurogenerative disorders. In contrast, previous research has highlighted zingerone's protective role in other brain regions like the hippocampus. The objective of this study is to investigate the effects of zingerone on the prefrontal cortex, an essential brain region responsible for planning, decision-making, and many higher-order cognitive tasks. Zingerone, a bioactive molecule produced from ginger, has been shown to penetrate the blood-brain barrier (BBB),

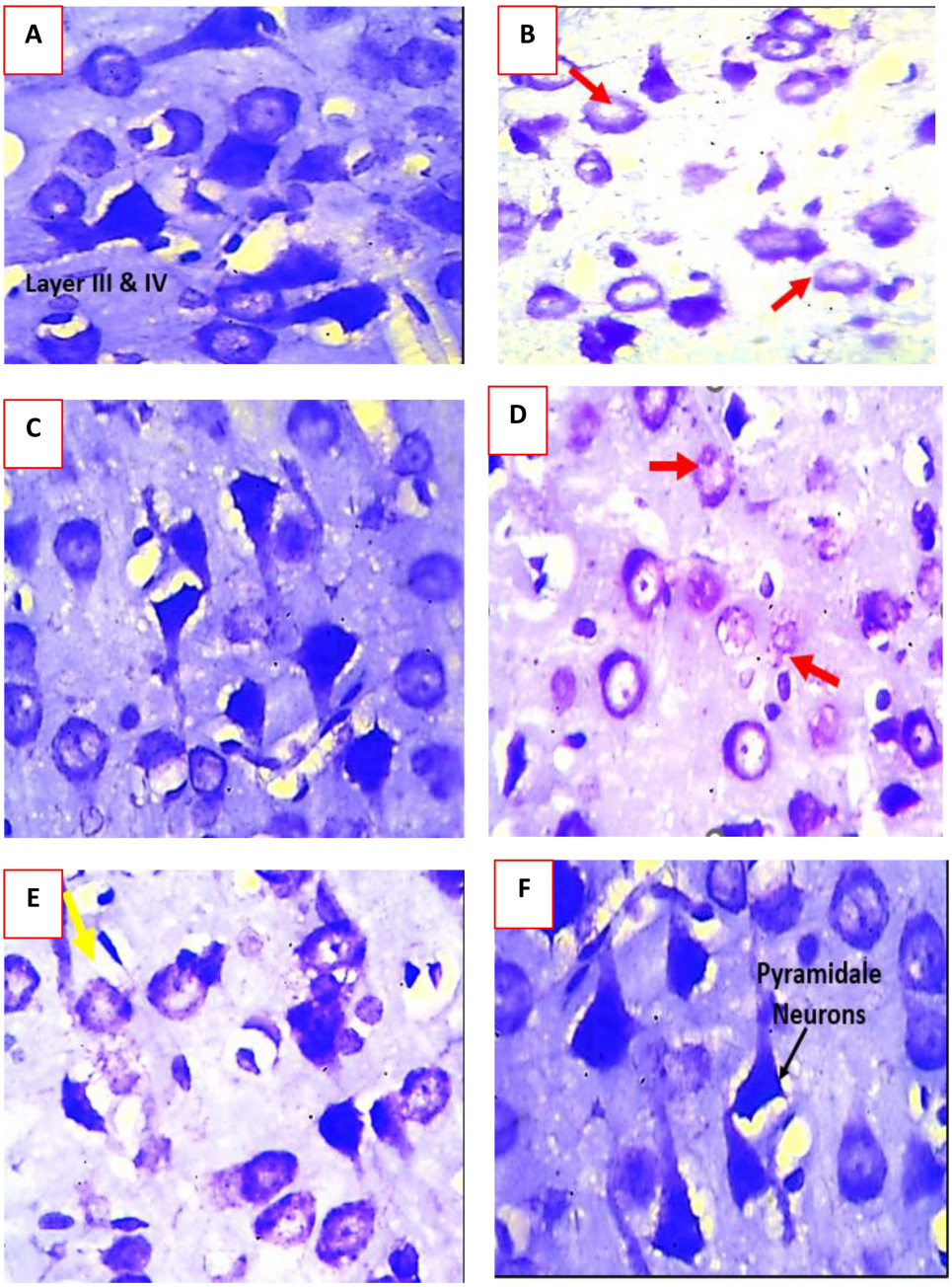

Figure 9 (**A**–**F**): Photomicrographs of the prefrontal cortex in rats from (Groups **A**–**F**), stained with Cresyl Fast Violet (Nissl stain) at ×400 magnification. (**A**) Normal cortical architecture with intact Nissl substance. (**B**) Diffuse neuronal distribution with chromatolysis, reduced Nissl substance, and loss of neuronal processes (red arrow), red arrow showing neuronal alteration (**C**) Normal histoarchitecture with preserved Nissl profiles and no signs of chromatolysis or pyknosis. (**D** and **E**) arrows (red and yellow) show mildly altered architecture showing slight neuronal dispersion and early chromatolysis. (**F**) Normal histology with intact Nissl substance and no evidence of neuronal damage, black arrow showing pyramidal neurons.

which is critical for its possible neuroprotective properties.[57,58] According to studies, zingerone may swiftly permeate the BBB and is digested efficiently in both rats and humans, reaching considerable quantities in the brain.[57,58] This permeability is required for zingerone to exert its antioxidant and anti-inflammatory properties within the central nervous system, particularly in the treatment of cadmium-induced neurotoxicity.[57,58]

By demonstrating that zingerone administration mitigates oxidative stress, reduces neuroinflammation and alleviates cognitive decline in the prefrontal cortex, this study contributes greatly to several research studies aimed towards the management of environmentally induced neurotoxicity.

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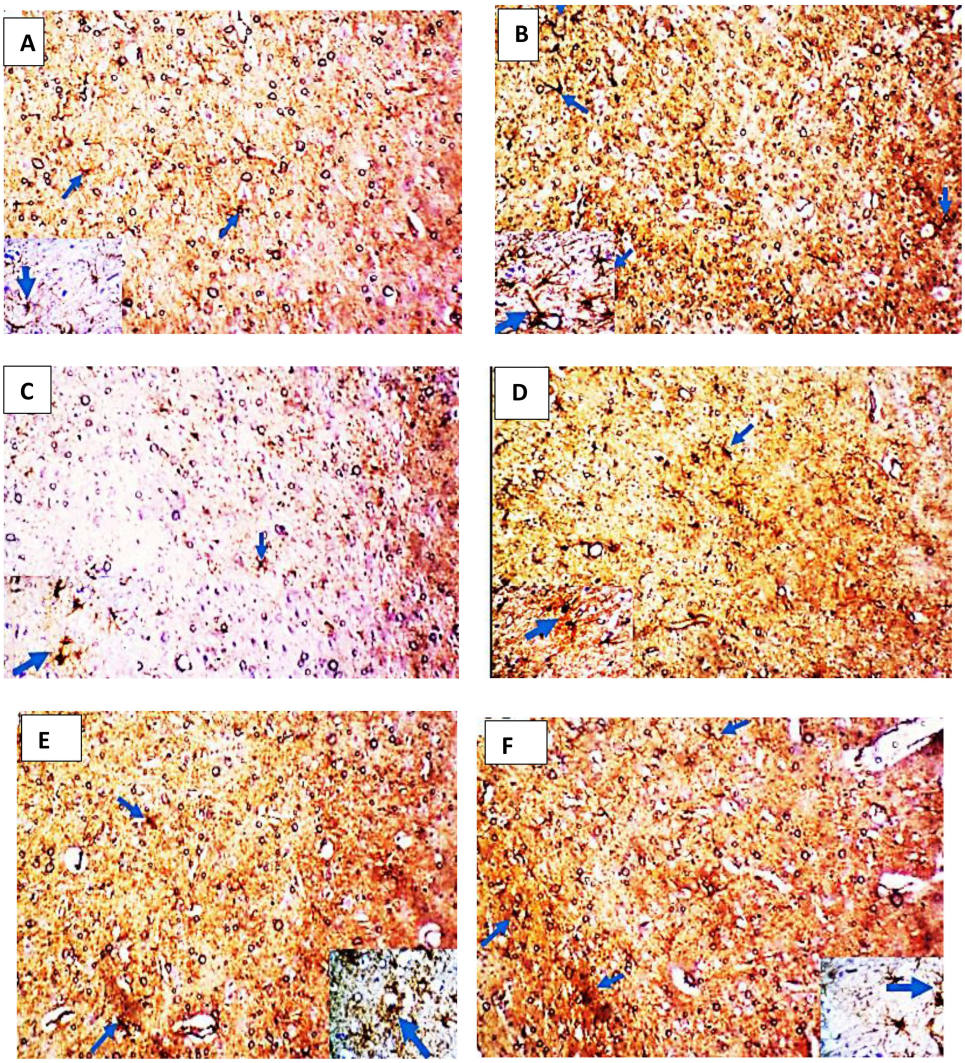

Figure 10 (**A**–**F**): Photomicrographs showing GFAP astrocytes activation by rat anti-GFAP antibody (scale bar 50um). (**A**) a significant decrease in astrocyte activity, (**B**) showed increased expressivity of astrocytes. In the treatment group (**C**–**F**), there was a significant decrease in Astrocytosis compared to the cadmium-only group. The dark brown colouration and blue arrow represent astrocyte activation, with group A and all zingerone-treated groups showing a decrease in astrocyte activation, while cadmium-only-treated only showed more intense staining, hypertrophied astrocytes and higher astrocyte density.

In the present study, we examined the effects of exposure to cadmium and zingerone treatment on the body weight of the experimental rats and discovered that all groups gained weight from the initial to the final measurements. However, the cadmium-only group gained minimal weight, demonstrating that cadmium hurts normal growth. While some reports show that cadmium reduced body weight, some other documented findings have demonstrated that cadmium administration can disrupt body weight gain. Heavy metals such as cadmium and zinc have been related to a decrease in body weight with evidence of tissue necrosis and apoptosis.[53,59,60] However, both the normal control and zingerone-only groups attained significant weight. Notably, rats treated with a combination of cadmium and zingerone (groups D-F) gained weight comparable to control groups, implying that zingerone inhibits cadmium-induced weight suppression and promotes normal growth patterns. This finding is in agreement with previous report.[60]

In this study, cadmium significantly decreased the object recognition ability of the experimental rats, as we observed a remarkable reduction in the average time for exploration of the novel object and the discrimination index. Rats treated with cadmium spent more time exploring familiar objects, suggesting impaired cognitive function. The development of neurodegenerative conditions like Parkinson's disease (PD) and Alzheimer's disease (AD) has been associated with heavy metal exposure, such as cadmium.[11,30,61] Cadmium-induced toxicity has been shown to target neurons in the cerebral

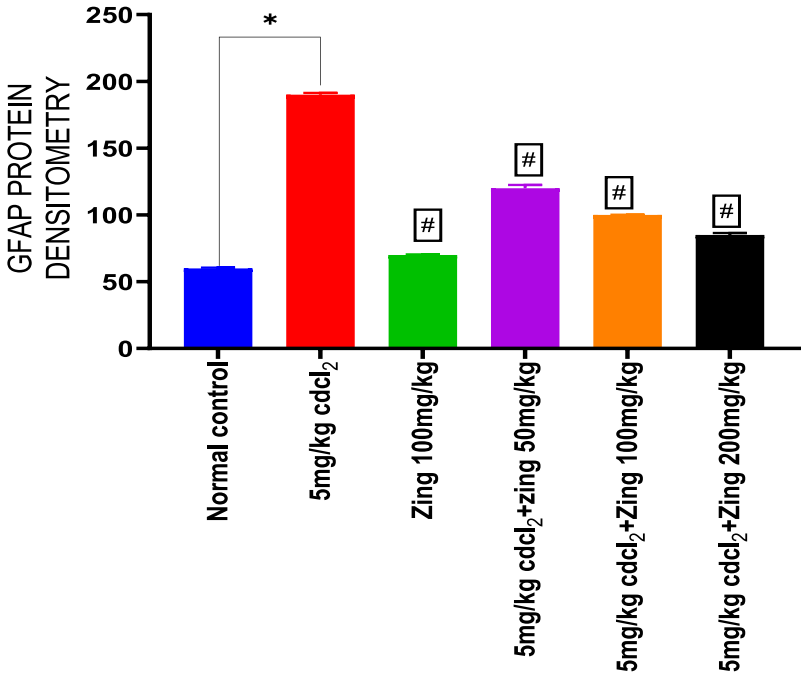

Figure 11 Bar charts showing Astrocyte (GFAP) expressivity in the prefrontal cortex across the groups using Image-J Software. *Statistically significant difference from the normal control group ($P < 0.05$), #Statistically significant difference in comparison to the cadmium only group ($P < 0.05$). Data expressed as Mean ± SEM. (n=5 rats).

cortex, leading to cell death.[52,53] Cadmium exposure led to observable behavioural changes, memory problems, and learning difficulties, affecting anxiety and fear responses as well as impairing memory. Prolonged exposure to cadmium has been associated with impair cognitive processes, specifically recognition memory as evaluated by the Novel Object Recognition Test (NORT), via pathways involving oxidative stress, neuroinflammation, and apoptosis.[18,62,63] By inhibiting mitochondrial respiration, cadmium (Cd) increases the generation of reactive oxygen species (ROS) and decreases ATP synthesis, both of which affect brain function.[18,62,64] Furthermore, Cd triggers inflammatory pathways by triggering pro-inflammatory cytokines release, leading to neuronal injury.[55] These combined effects cause neuronal death, notably in the prefrontal cortex and hippocampus, a brain area important for memory functions assessed by NORT. Finding demonstrates that both morphological neuronal alterations and cholinergic dysfunction contribute to a decline in NOR performance and subsequent cognitive decline.[23,30,55]

However, treatment with *zingeron*e resulted in notable cognitive improvements, including increased novel object recognition time and a higher discrimination index across all *zingerone*-treated groups.[16] Zingerone regulates apoptotic pathways, preventing Cd-induced neuronal cell death. Zingerone protects brain function and enhances cognitive outcomes via these processes, as demonstrated by NORT. As mentioned earlier, these behavioural deficits are likely related to increase oxidative damage and neuroinflammatory indicators in the brain.[53] Inflammation is strongly associated with various pathological conditions, including Alzheimer's disease and chronic cognitive impairment.[65] Increase in key indicators of inflammation like IL-6 and TNF-α, may impair the function of neurons and increase the risk of oxidative stress-related neuronal apoptosis.[30,43] While these cytokines support long-term plasticity, learning, and memory under normal physiological conditions, elevated levels of cytokines, as seen in brain injury models, can negatively impact memory mechanisms, behaviour, and homeostatic plasticity.[66]

Our result also shows that cadmium exposure induced elevated levels of *IL-6 and TNF-α*, which is consistent with previous findings.[61,67] Cadmium-induced toxicity was associated with increased pro-inflammatory markers, indicating tissue damage. *zingerone* treatment, however, significantly reduced IL-6 and TNF-α levels across all *zingerone*-treated groups compared to the cadmium-only group. This aligns with other studies, indicating that *zingerone* has anti-inflammatory properties.[35,68] Inhibition of neurogenesis in the hippocampus and cortical regions, neuronal degeneration, and cognitive impairment have all been connected to cadmium toxicity[18,62] Cadmium exposure has been reported to

generate reactive oxygen species (ROS), which can lead to lipid peroxidation, DNA damage, disruption of cells' antioxidant defences, brain toxicity, and cognitive impairment.[43,45] Findings from this study shows that treatment of 5mg/kg of cadmium caused reduction in endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase and brought up the level of MDA, this is agreement with.[18,23,41,46,69,70]

Our findings revealed that zingerone mitigates oxidative stress by scavenging free radicals and improving natural antioxidant enzymes activity including catalase (CAT) and superoxide dismutase (SOD). Furthermore, zingerone reduces malondialdehyde (MDA) level, an indicator of lipid peroxidation, thereby protecting the cellular membrane from damage. These findings are in agreement with previous studies.[32,35,42] The chelating qualities of zingerone might also help decrease the buildup of cadmium in tissues, which could mitigate its harmful effects significantly and this is also seen in other neuroprotective medicinal plant.[71]

Histopathological findings revealed that different dosages of zingerone were effective in reversing cadmium-induced neurogenesis and cortical alterations. The prefrontal cortex in rats treated with *zingerone* showed reduced degenerative changes, including fewer pyknotic neurons and less cytoplasmic fragmentation, compared to the cadmium-only group. Our observations agree with earlier reports on the histopathological effects of cadmium on the prefrontal cortex.[64,72]

The higher dose of *zingerone* demonstrated substantial therapeutic potential in mitigating cortex toxicity and cognitive impairment, corroborating previous research highlighting *zingerone's* neuroprotective properties.[30,39] GFAP staining in immunohistochemical analysis demonstrated that the group exposed to cadmium alone demonstrated greater astrocyte activity compared to the groups that received zingerone and the normal control. Astrocytosis, also known as reactive astrogliosis, is an aberrant increase in astrocyte numbers caused by damage to the central nervous system (CNS), such as trauma, infection, ischemia, or neurodegenerative disorders.[73] Astrocytes respond to neuronal damage by undergoing morphological and functional changes, like as hypertrophy and proliferation, to maintain homeostasis and protect neural tissue. They contribute by generating glial scars, which isolate injured areas, releasing neurotrophic substances, and regulating inflammation. However, severe or chronic astrocytosis may stunt brain healing by blocking axonal regrowth and producing neurotoxins.[73,74] Astrocytosis plays a dual role in the course of neurological disorder such as Alzheimer's disease, Parkinson's disease, and multiple sclerosis.[73] Immunohistochemical examination using Glial fibrillary acidic protein (GFAP) staining of the prefrontal cortex showed an observable increased astrocyte activity in groups that received cadmium only, compared to normal control and *zingerone-treated* groups. Elevated GFAP expression is indicative of chronic inflammation and neuronal damage caused by cadmium.[53] Administration of zingerone significantly decreased GFAP levels, indicating that it is effective in reducing astrocyte activation and related neuroinflammation.

Conclusion

In conclusion, findings from this study shows that cadmium induced neurotoxicity by promoting oxidative stress, inflammation, astrocytosis and cognitive decline. However, treatment with zingerone reduces cadmium-induced cognitive impairment by influencing important neural pathways. The therapeutic potentials of zingerone was greatly demonstarted by the reduction of oxidative stress, astrocytes expressivity and neuroinflammation, as well as the preservation of prefrontal cortex structure. The findings imply that zingerone has therapeutic potential for alleviating heavy metal-induced neurotoxicity.

Abbreviations

GFAP, Glial Fibrillary Acidic Protein; AD, Alzheimer's Disease; PD, Parkinson's Disease; CNS, Central Nervous System; PFC, Prefrontal Cortex; IL-6, Interleukin-6; H&E, Hematoxylin and Eosin; CFV, Cresyl Fast Violet staining; CD, Cadmium; NORT, Novel object recognition test; TNF-α, Tumor necrosis factor alpha; SOD, Superoxide dismutase; CAT, Catalase; MDA, Malondialdehyde; Zing, Zingerone; BBB, Blood Brain Barrier.

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Author Contributions

Each author contributed significantly to this study, in the area of conception, study design, execution, data collection, analysis, and interpretation. All author participated in the article's drafting, revision, or critical review and approved the final version before it was published. All author agreed on the journal to which the article was submitted and take responsibility for every part of the work.

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Disclosure

The authors declare that they have no competing interests.

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